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Chronoamperometrically poised electrodes mimic the performance of yeast-based bioanode in MFC

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Poised electrodes in a three-electrode mode is recently used as an alternative tool for establishment of favorable redox conditions for extracellular electron transfer (EET) from biocatalysts to the anode in bioelectrochemical systems. It has been demonstrated that the optimal imposed potentials differ for a particular microorganism species, depending on its specific metabolic pathways. In this study, carbon felt electrodes were potentiostatically poised at +0.005, +0.405, +0.505 and + 0.605 V (vs. SHE) in the presence of exoelectrogenic yeast strain *Candida melibiosica* 2491. The results from chronoamperometric experiments show that the yeast is capable of performing EET at potentials higher than +0.5 V. The current generated at + 0.605 V (vs. SHE) follows the yeast growth phases, reaching stable maximal outputs of ca. 40 mA/m^2. The cyclic voltammetry analysis carried out reveals that the observed electrochemical activity is due to production and secretion of endogenous mediator of EET, mimicking the already established yeast performance in real biofuel cells. The differences in the electrochemical impedance spectra obtained with exploited yeast suspension and cellular-free fraction describe the contribution of the cellular processes to the anodic current generation.

Key words: microbial fuel cell mimicking, poised anode, endogenous mediator, chronoamperometry, cyclic voltammetry, electrochemical impedance spectroscopy.

INTRODUCTION

The oxidation–reduction processes are the basis of the cellular energetic mechanism. The most energy-efficient oxidation of a substrate by living microorganisms occurs at terminal electron acceptor possessing high positive potential like the oxygen. Under anaerobic or semi-aerobic conditions, the aerobic respiration processes are partially replaced by alternative pathways such as different kind of fermentations. Although less energetic favorable these pathways contribute to the equilibration of the intracellular redox cell balance providing oxidized NAD necessary as a cofactor of glycerinaldehyd-3-phosphat-dehydrogenase and thus for further conduction of glycolysis. The capability of using alternative electron acceptors is at the heart of Microbial fuel cell (MFC) technology development, where the intracellular produced electrons are transferred extracellularly to the anode serving as a final electron acceptor [1]. The bacteria utilizing the anode as electron acceptor are referred to as exoelectrogens or anode-respiring bacteria [2].

To gain these electrons (in form of electrical energy), the MFC-devices usually operate under polarization conditions, where the anode and the cathode are connected in an electrical circuit [3]. At a relatively constant cathodic potential during the operation, the lower the anodic potential, the higher the electrical outputs. Reaching anodic potential of ca. -0.3 V (vs. SHE) is reported as most thermodynamic profitable. This potential can be reached, however, for longer time at flow-batch operating bioelectrochemical systems (BES). The formation of microbial biofilm on the anodic surface leads to lowering the potential [4]. Having in mind the direct proportional relation between microbial metabolic activity and the generated electrical current, an alternative method for feeding potential (poised electrode) has been proposed [5-7]. From theoretical point of view, a more positive anode potential wil allow the cell capture more energy, but only if the cell is capable of capturing this energy by pumping additional

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protons across its cellular membrane.

Thus, the microbes must possess terminal proteins of respiratory chains that can use this additional potential provided by the anode [8]. Marsili et al. support this opinion by giving the following example [9]: If the anode of an acetate-fed (Eo′= -0.3 V vs. SHE) MFC is set to a highly positive value of +0.4 V and a microbe can only adjust its respiratory enzymes to a lower potential, e.g., Eo′enzyme = -0.05 V, then the microbe will only be able to capture a part of the total available free energy.

Set anode potentials in MFC differ widely from -0.2 V to +0.8 V (vs. SHE). It is accepted that the anode potential has to be more positive than that of the potential of the substrate and thus to simulate natural terminal electron acceptors. The use of different electrode materials such as stainless steel or graphite does not allow the comparison of the current generated at poised anodes. In their review article Wagner et al. [8] summarize the received data and show that current densities generated at different applied potentials have very different values. Some investigators suggest potential input at negative potentials, which would create conditions similar to those in a MFC. Others suggest that more positive potentials would provide more free energy to the microorganisms. Depending on the microorganisms used as biocatalysts, the optimal anode potentials, leading to high current densities and more rapid start-up times, needs further investigation.

In this study, the influence of the imposed potential on the extracellular electron transfer (EET) from *Candida melibiosica* 2491 yeast to carbon-felt electrode was examined. For this purpose, chronoamperometry (CA) with different imposed potentials between -0.2 and +0.4 V vs. Ag/AgCl was applied to the electrode, immersed in an acetate buffer, where yeast cells had been suspended for cultivation. The behavior of the culture and the cell response to the applied electrical voltage was explored by means of cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS).

EXPERIMENTAL

Yeast cultivation

The yeast inoculum was fresh prepared after *C. melibiosica* 2491 was cultivated at enriched medium (YP_{fru}) as previously described [10]. 500 ml suspension was centrifuged at 5000xg and washed twice with acetate buffer. 6% inoculum with *Candida melibiosica* was determined by spectrophotometric measurement at A_{600}=0.630 at 100x dilution. The biocatalyst had been sterile inoculated in 40 ml 0.2 M acetate buffer, pH 4.6, allowing 3 h adaptation before the respective potential was applied.

Electrochemical analyses

Potentiostatically-controlled anodes at four different potentials (-0.2, +0.2, +0.3, +0.4 V vs. Ag/AgCl) have been examined for current generation for 50 h in a batch operation mode. At each particular potential, the experiment was carried out separately, however, the conditions have been unified (electrode surface, quantity of inoculum, temperature, electrolyte). The analyses have been performed in a three-electrode mode, where a carbon felt (SPC-7011, 30 g/m^2, Weissgerber GmbH & Co. KG) with geometric area of 16 cm^2 was connected as a working, Pt-wire as a counter and Ag/AgCl (3.5 M KCl) as a reference electrode. The experiments were carried out at 26 °C. The generated current was monitored during the whole experimental window and recorded every five minutes by PalmSens 3 potentiostat. For better comparison, the received data are presented as unified current density (mA/m^2) and the applied potentials referred to SHE (Table 1).

Table 1. Applied potentials to the working electrode in the presence of yeast suspension.

Half-cell samples	Poising potentials, V vs. SHE
$Anode_1$	+0.005
$Anode_2$	+0.405
$Anode_3$	+0.505
$Anode_4$	+0.605

The performance of yeast biocatalyst has been also examined by means of cyclic voltammetry (CV) for determination of the redox activity of the yeast suspension and electrochemical impedance spectroscopy (EIS) for clarification of the charge transfer hindrances after the anode poisoning. EIS was carried out in the frequency range from 50 kHz to 5 mHz with an applied ac signal with an amplitude 10 mV and Edc equal to characteristic peak potentials in CVs.

At the end of the chronoamperometric experiments the yeast suspension was collected and yeast cells were pelleted by centrifugation at 5000xg for 10 minutes. The obtained cellular-free anolyte was filtrated by sterile filter with 0.2 μm ϕ

pores` size. The electrochemical activity of the filtrate was analyzed by CV and EIS.

RESULTS AND DISCUSSION

Anodic set potentials have been chosen to be sufficiently positive so that to allow the use of the anodes for extracellular transfer of electrons by the yeast cells (Fig. 1). At +0.005 V (vs. SHE) the current began from too negative values, rising fast in the first hour, but remaining negative till the end of experiment (Fig.1a). At +0.405 V (vs. SHE) the response of the yeast in the beginning differed but still manifested in negative currents. These results show definitely that both potentials are insufficient to force the yeast cells to transfer electrons/protons to the anode. Increasing the set potentials up to +0.605 V (vs. SHE) (Fig.1b) leads to positive current densities, which slightly decreased at +0.505 V, but increased at +0.605 V. The best performance was obtained by poising at +0.605 V (vs. SHE). The resulting curve resembles the growth curve of the culture, previously established [10]. Relative stable current density of ca. 40 mA/m2 has been achieved.

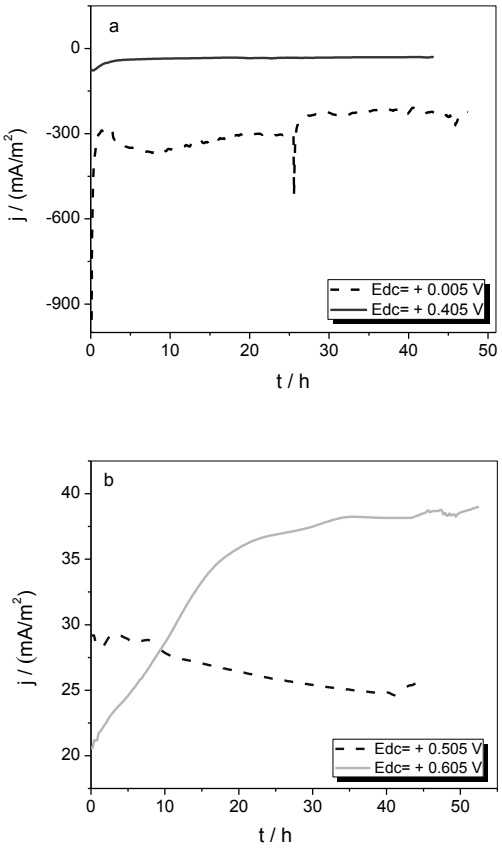

Fig. 1. Current densities over time with anodes set at: a) +0.005 V (dashed line) and +0.405 V (solid line) potentials; b) +0.505 V (dashed line) and +0.605 V (solid line) vs. SHE.

Having in mind that at the end of exponential phase of growth the electrogenic properties of *C. melibiosica* are characterized with the production of exogenous mediator (EnM), we carried out CV for tracing the redox activity of the yeast culture at the different set of potentials applied. The results are presented in Fig. 2.

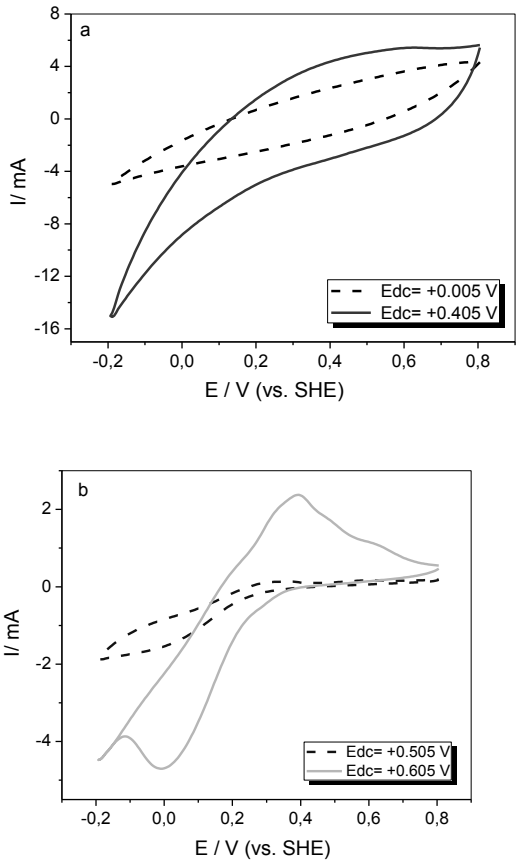

Fig. 2. CV of yeast suspension cultivated at applied potentials: a) +0.005 V (dashed line) and +0.405 V (solid line) potentials; b) +0.505 V (dashed line) and +0.605 V (solid line) vs. SHE. Scan rate 10 mV/s.

As expected, none visible redox peaks have been observed with the electrodes poised at +0.005 and +0.405 V (vs. SHE) (Fig. 2a). Unlike them, the cyclic voltammogram of the electrode poised at +0.505 V (vs. SHE) reveals slightly hinted peaks at +0.335 and +0.595 V (vs. SHE), respectively (Fig. 2b). The registered oxidation peaks directs that the yeast are electrochemically active around this potential range. A significant augmentation of yeast redox activity is observed after poising the anode at +0.605 V (vs. SHE). Both anodic and cathodic currents have been enlarged with a clearly distinguishable oxidation peak at +0.395 V and a reduction peak at -0.010 V (vs. SHE).

The yeast capability of secreting an EnM participating in electricity generation processes

with $Anode_4$ was additionally analyzed by both CV and EIS. The recorded CVs are characterized by appearance of broad anodic and cathodic peaks, which potential difference rises with the increase of scan rate, indicating a quasi-reversible electrochemical behavior (Fig. 3a). The estimated formal redox potential ($E^{o`}$) from the CV obtained at 10 mV/s is +0.195 V (vs. SHE), which is close to data from our previous studies carried out in real MFCs [2], in which the formal redox potential of non-fractionated yeast suspensions ranges between +0.200 and +0.285 V (vs. SHE) depending on the substrate used [12].

The linear dependence of the peaks` currents on the square root of scan rates reveals a presence of electroactive soluble compound, capable of transferring electrons from the yeast cells to the anode (Fig. 3a, inset). The impedance data (Fig. 3b) fit well to R_1-CR_2-QR_3 equivalent electrical circuit model. Because the second arc is not an ideal semi-circle but rather a depressed one, a constant phase element (CPE) was introduced instead of a second capacitor.

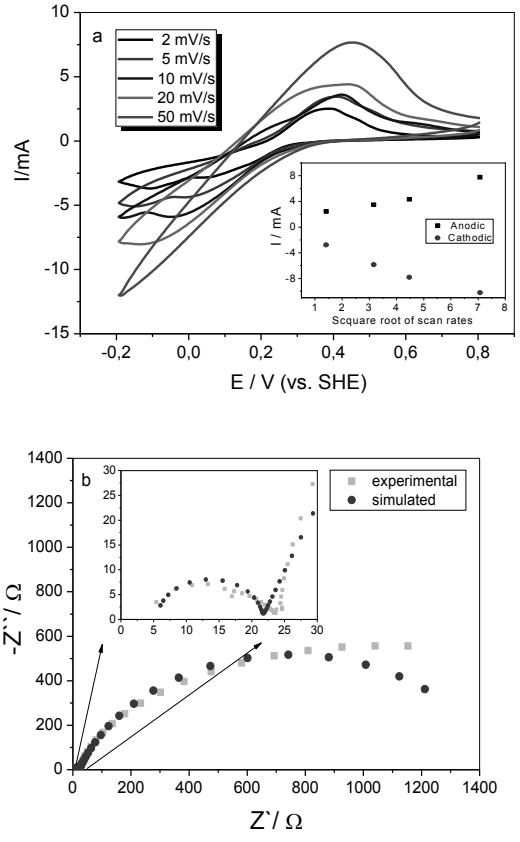

Fig. 3. Electrochemical analyses of yeast suspension after chronoamperometric experiments at +0.605 V (vs. SHE): a) CV with different scan rates. Inset: Dependence of anodic and cathodic peak currents on the square root of scan rate.; b) EIS-Nyquist plot – E_{ac}=10mV, E_{dc}= $E_{ox\ peak}$.

The results show that the ohmic resistance (R_1 = R_Ω = 5.5 Ω) and the charge transfer resistance (R_2 = R_{CT} = 16 Ω) are insignificant compared to R_3= 1455 Ω. Considering the presence of living cells, their redox activity and therefore the complexity of the system, the second arc might be attributed to internal cellular metabolic processes, as proposed by Sekar and Ramasamy [11], and the resistance R_3, denoted as R_{in}, is determined by the yeast ability to grow under these conditions and to produce electrochemically active compounds to communicate with the anode. It is considered that the in parallel connected capacitance and the resistance of the first arc describe the external processes, occurring on the electrode surface (that is why R_2 is referred to as R_{ex}) with the contribution of the biological elements (whole cells and secondary metabolites).

To distinguish the contribution of the yeast cells themselves from that of the secreted active metabolites to the current generation processes, the exploited anolyte was fractionated and the redox activity of the cellular-free supernatant was investigated by CV (Fig. 4a). The results show the presence of a soluble redox active substance with a formal potential $E^{o`}$= +0.192 V (vs. SHE). Assuming that all molecules secreted by the yeast cells are in a reduced form to complete their biological role for extracellular electron transfer to the anode, at a positive potential of +0.217 V (vs. SHE) the molecules contacting the electrode surface have been electrochemically oxidized, resulting in a sharp oxidation peak. Sweeping the potential in negative direction leads to their reduction and a formation of a cathodic peak with a maximum at +0.166 V (vs. SHE). Comparing the peak potentials obtained with cellular-free fraction and yeast suspension at the same scan rate (Fig 3a), it is seen that in the presence of yeast cells, the anodic peak is shifted in positive direction, while the cathodic peak is shifted to more negative values. The peak currents show with an order of magnitude higher values, which in turn directs to an enhanced oxidation ability in the presence of the yeast biocatalysts. Within the cells, EnM is reduced by NADH or $FADH_2$ (cofactors of important enzymes) in the cytoplasm or by cytochromes of the mitochondrial electron transport chains [14].

The impedance spectra also showed significant difference in comparison with the yeast suspension behavior (Fig. 4b). The lack of a second arc at the absence of yeast cells supports the hypothesis that this arc describes the hindrances connected with the

complex cellular processes contributing to the extracellular electron transfer (EET). Having in mind that in the yeast suspension, the oxidized EnM enters the cells to be reduced again and secreted in the medium to be re-oxidized on the anode repeatedly, the lack of yeast cells is responsible to the huge charge transfer resistance in the cellular-free fraction. The Bode plot data, however, show a maximum negative phase angle at mid frequencies (Fig. 4b - inset), which is attributed to the presence of EnM, as previously suggested [11].

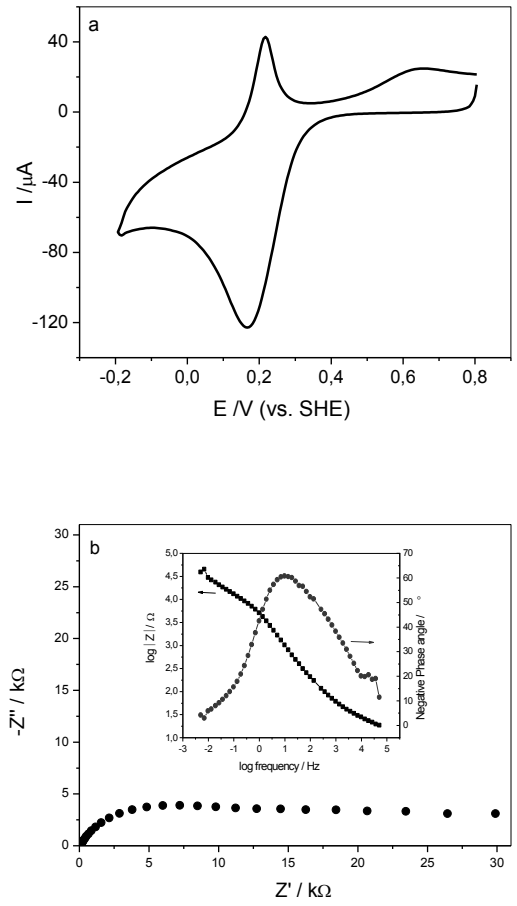

Fig. 4. Electrochemical analyses of filtrated cellular-free fraction, containing EnM: a) CV, scan rate 10 mV/s, second scan; b) EIS-Nyquist plot (Eac=10mV, Edc= Eox peak). Inset: EIS-Bode plot.

As stated by Wagner et al. [8], more positive anode potentials should allow microorganism to gain more energy per electron transferred than a lower potential, but this can only occur if the microbe has metabolic pathways capable of capturing the available energy. In our recent study [15], we proved that in the presence of acetate *C. melibiosica* yeast up-regulates the Glyoxylate cycle for acetate assimilation and biosynthesis of carbohydrate precursors such as malate, succinate and oxaloacetate.

The investigation was carried out in real two-chamber MFCs with continuously connected external load. The obtained results in this study reveal that +0.605 V (vs. SHE) is an appropriate potential to mimic MFC-conditions mentioned above, while the yeast use acetate as a substrate. This potential is sufficient also to up-regulate a secondary metabolic pathway for synthesis of active metabolite (EnM), contributing to the current generation under these conditions.

CONCLUSIONS

The half-cell studies with potentiostatically poised carbon felt electrodes and *C. melibiosica* 2491 yeast as a biocatalyst show that anodic reaction connected with the yeast electrochemical activity occurs at potentials higher than +0.5 V (vs. SHE). The current density at +0.605 V (vs. SHE) reached stable values of ca. 40 mA/m^2 in a batch operation mode. After reaching a steady-state, well-defined anodic and cathodic peaks appear in the cyclic voltammograms of the anolyte, indicating a production of endogenous mediator for accomplishment of extracellular electron transfer. The differences in the impedance spectra obtained in the presence and absence of yeast cells reveal the contribution of the cellular processes to the EET and the anodic current generation.

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Хроноамперометрично приложено напрежение върху електрод имитира поведението на дрожден биоанод в биогоривна клетка

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(Резюме)

Подаването на напрежение на електроди в три електродна електрохимична клетка се използва като подход за установяване на благоприятни редокс-условия за извънклетъчен електронен трансфер (EET) от биокатализатора до анода. Демонстрирано е, че оптималните приложени потенциали се различават за отделните видове микроорганизми и зависят от специфичните им метаболитни пътища. В това изследване, потенциали от +0.005, +0.405, +0.505 и + 0.605 V (срещу SHE) са прилагани върху въглеродни електроди в присъствието на екзоелектрогенен щам на дрожди *Candida melibiosica* 2491. Резултатите от хроноамперометричните експерименти показват, че дрождите са способни да осъществяват EET при положителни потенциали, по-високи от +0.5 V. Токът, генериран при + 0.605 В (срещу SHE), следва фазите на растеж на дрождите, достигайки стабилни максимални нива от 40 mA /m^2. Извършената циклична волтамперометрия показва, че наблюдаваната електрохимична активност се дължи на произведен и секретиран ендогенен медиатор на EET, имитирайки вече установеното поведение на дрождения щам в реални биогоривни клетки. Разликите в електрохимичните импедансни спектри, получени със дрождена суспензия и безклетъчни фракции, описват приноса на клетъчните процеси към генерирането на аноден ток.